Data Evaluation Report on the 28-day Sub-chronic Toxicity of Rimon Technical to the Rainbow Trout (*Oncorhynchus mykiss*).

PMRA Submission Number{.....} EPA MRID Number 45638216

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D285479
	OECD Data Point	
	EPA MRID	45638216
	EPA Guideline	N/A (OECD Guideline No. 204)

Test material: Rimon Technical **Purity**: 99.3%
Common name: Novaluron
Chemical name: IUPAC: 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea
CAS name: N-[[[3-Chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]carbonyl]-2,6-difluorobenzamide
CAS No.: 116714-46-6
Synonyms: None specified

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

QC Reviewer: Christie E. Padova, B.S.
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

Primary Reviewer: Bill Evans, Biologist
OPP/EFED/ERB - I

Date: 11/26/03

Reference/Submission No.

Company Code:
Active Code:
EPA PC Code: 124002

Date Evaluation Completed:

CITATION: Jenkins, C.A. 1998. "RIMON" Technical, Prolonged Toxicity to Rainbow Trout Under Flow-Through Conditions, 28-Day Study. Unpublished study performed by Huntingdon Life Sciences Ltd., Eye, Suffolk, England. Laboratory Project Identification No. MAK/441/974266. Study submitted by Makhteshim Chemical Works Ltd., Beer-Sheva, Israel. Study initiated September 15, 1997 and completed May 18, 1998.

EXECUTIVE SUMMARY:

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{EPA/OECD/PMRA}
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EXECUTIVE SUMMARY:

In a 28-day sub-chronic toxicity study, 84-day-old Rainbow trout (*Oncorhynchus mykiss*) were exposed to Rimon Technical (Novaluron) under flow-through conditions at nominal concentrations of 0 (negative and solvent controls) and 10 μg/L (limit test based on aqueous solubility of 3.0 μg/L). The mean-measured concentrations were <0.25 (<LOD;, controls) and 6.16 μg a.i./L.

No mortality was observed in either the control groups or the 6.16 μg a.i./L test group. The 28-day LC_{50} value was therefore >6.16 μg/L. In addition, no treatment-related signs of toxicity or effects on terminal growth (fork length and wet weight) were observed. The NOEC for sub-lethal effects was therefore 6.16 μg/L, and the LOEC was >6.16 μg/L.

The study is scientifically sound; however, it was not designed to fulfill any current U.S. EPA FIFRA guideline. This study is therefore classified SUPPLEMENTAL, as it provides useful information on the 28-day sub-chronic toxicity of Novaluron to the Rainbow trout (*Oncorhynchus mykiss*).

Results Synopsis:

Test Organism Size/Age (mean Weight or Length): Juvenile, 84 days old; mean length of 5.7 cm and mean wet weight of 2.1 g (n=10)
Test Type (Flow-through, Static, Static Renewal): Flow-through

28-Day Survival:
LC_{50}: >6.16 μg a.i./L 95% C.I.: N/A
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

Toxic Effects:
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

28-Day Wet Weight:
NOEC: >6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

28-Day Length:
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

Most sensitive endpoint(s): None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol was based on OECD Guideline No. 204 for testing of chemicals, "Fish Prolonged Toxicity Test: 14-day Study" (1984). This study was not designed to fulfill any current U.S. EPA FIFRA guideline. General deviations from FIFRA guidance involving acute

(§72-1c) and/or early life stage (§72-4a) toxicity studies with Rainbow trout included:

1. This study was conducted as a limit test (based on aqueous solubility), so only one concentration was tested.

2. The hardness and pH levels of the dilution water exceeded recommended ranges for acute trout studies.

These deviations do not affect the scientific validity of the study. This study was not designed to fulfill any current U.S. EPA FIFRA guideline.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards set forth by the United Kingdom GLP Compliance Program (1997), EC Council Directive (1986), OECD (1997), U.S. EPA (1989) and the JMAFF (1984).

A. MATERIALS:

1. Test Material Rimon Technical (Novaluron)

Description: Fine, purple-tinted powder

Lot No./Batch No. : 970211/4

Purity: 99.3%

Stability of Compound Under Test Conditions: Although recoveries were consistently low, Rimon Technical was stable in the unfiltered test water, ranging from 51.5 to 67.9% of the nominal concentration (excludes Day 0 samples; Table 1, p. 19).

Water solubility: 3.0 μg/L

OECD requires water solubility, stability in water and light, pK_a, P_{ow} and vapor pressure of the test compound. Aside from aqueous solubility, OECD requirements were not reported.

Storage conditions of test chemicals: Ambient conditions, protected from light.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss)*

Size/Age: Juvenile, 84 days old; mean length of 5.7 cm and mean weight of 2.1 g (n=10)

Source: Parkwood Trout Farm, Kent (reared at Gadewater Trout Farm, Hemel Hempstead from South African eggs).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None conducted. The test level was selected based on the reported limit of aqueous solubility (3.0 μg/L).

b. Definitive Study:

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	14 Days Same as test Trout crumb (TROUW UK (Ltd) Nutra Trout Fry 02), 2 to 4% of total fish wet-weight was provided daily. Less than 1% mortality was observed during the 14 days of acclimation.	
Duration of the test	28 Days	
Test conditions: static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal:	 Flow-through Continuous-flow diluter; 9.6 test vessel volumes/24 hours; the operation of the dosing apparatus was confirmed. N/A	The dosing apparatus failed and was restarted on Day 10 of the test.
Aeration, if any	None	

Parameter	Details	Remarks *Criteria*
Test vessel Material: Size: Fill volume:	 Glass aquaria 15 L 10 L (13-cm depth)	
Source of dilution water	Tap water was filtered through active carbon to remove chlorine and softened with reverse-osmosis treated tap water. Water was gently aerated prior to testing.	The water was periodically analyzed for chemical contaminants and the presence of microbes; results are provided on Appendix 2, p. 22.

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 48-59 mg/L 7.0-7.9 69-102% 13.4-14.2°C 0.8-0.9 mg/L Not specified See Appendix 2, p. 22 Not detected Not specified	In acute Rainbow trout studies (FIFRA §72-1c), EPA requires a water hardness of 40-48 mg/L as $CaCO_3$ and a pH range of 7.2-7.6. Temperature, DO, and pH were measured daily in each aquarium; hardness was determined weekly in each aquarium.
Concentration of test material: nominal: measured:	 0 (negative and solvent controls) and 10 μg/L <0.25 (<LOD; controls) and 6.16 μg a.i./L	Samples were collected and analyzed on Days 0, 1, 7, 14, 21, and 28. Mean-measured concentrations were from unfiltered analytical data and excluded Day-0 data, which was slightly higher than the remaining measurements (Table 1, p. 19).
Solvent (type, percentage, if used)	Dimethylformamide (DMF), 0.1 mL/L	
Number of fish/replicates: negative control: solvent control: treated:	 20 fish: 10 fish/replicate, two replicate chambers 20 fish: 10 fish/replicate, two replicate chambers 20 fish: 10 fish/replicate, two replicate chambers	
Biomass loading rate	0.21 g/L/day	

Parameter	Details	Remarks *Criteria*
Lighting	16 hour light:8 hour dark photoperiod, with a transition period	Light intensity was 309 lux.
Feeding	During the test, fish were fed fish food (TROUW (UK) Ltd, Nutra Trout Fry 02), 2% of initial total fish wet-weight was provided daily.	
Stability of chemical in the test system	Although recoveries were consistently low, Rimon Technical was stable in the unfiltered test water, ranging from 51.5 to 67.9% of the nominal concentration (excludes Day 0 samples; Table 1, p. 19).	Recoveries of centrifuged samples ranged from 16.2 to 44% of the nominal concentration.
Recovery of chemical Level of Quantitation Level of Detection	51.5-67.9% of nominal Not specified 0.25 μg/L	Based on unfiltered analytical recoveries.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Survival, length, wet weight, and sublethal effects (toxic signs).	

Observation intervals	Mortality and sublethal effects were measured daily. Length and wet weight were measured at test initiation (n=10) and test termination.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No mortality was observed in the controls or the 6.16 μg a.i./L test group. The NOEC for mortality was 6.16 μg a.i./L.

Table 1: Effect of Rimon Technical on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, μg a.i./L, measured and (nominal conc.)	No. of fish at start of study	0-10 Days		11-20 Days		21-28 Days	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	20	0	0	0	0	0	0
Solvent control	20	0	0	0	0	0	0
6.16 (10)	20	0	0	0	0	0	0
NOEC	6.16 μg a.i./L						
LC_{50}	>6.16 μg a.i./L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

Hyperventilation and/or aggression were observed during the test in the controls and treatment group. The symptoms were not sustained and were not thought to be significant nor to have affected the integrity of the test. It was noted that all fish from the test and control groups fed actively throughout the test.

No treatment-related effect on terminal (Day 28) growth was observed between the test and solvent control group. The NOEC for both growth endpoints was 6.16 μg a.i./L.

Table 2. Effect of Rimon Technical on growth of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, μg a.i./L, measured and (nominal conc.)	Length (mm)	Wet weight (g)
Negative control	66	3.41
Solvent control	68	3.60
6.16 (10)	66	3.49
NOEC	6.16 μg/L	6.16 μg/L
LOEC	>6.16 μg/L	>6.16 μg/L
MATC	Not determined	Not determined

C. REPORTED STATISTICS:

The ANOVA analysis of variance test was performed to evaluate the body weight and body length measurements. The LC_{50} and EC_{50} values were not calculated. The NOEC and LOEC values were determined by a visual inspection of the data. Mean-measured concentrations were used in all estimations.

D. VERIFICATION OF STATISTICAL RESULTS:

The NOEC and LOEC for mortality, toxic effects, and fork length could be visually determined. The negative control and solvent control for wet weight were compared using a Student's t-test and upon finding no difference, the two were pooled for comparison to the treatment group. A Student's t-test detected no difference between the pooled control and treatment group for this endpoint. Mean-measured concentrations were used in all estimations.

28-Day Survival:
LC_{50}: >6.16 μg a.i./L 95% C.I.: N/A
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

Toxic Effects:
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

28-Day Wet Weight:
NOEC: >6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

28-Day Length:
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

Most sensitive endpoint(s): None

E. STUDY DEFICIENCIES:

Currently, there is no U.S. EPA requirement or guidance for a sub-chronic (28 day) freshwater fish toxicity study. The study deficiencies did not affect the scientific validity of the study, and therefore, this study is classified SUPPLEMENTAL because it provides useful information on the 28-day sub-chronic toxicity of Rimon Technical (Novaluron) to the Rainbow trout (*Oncorhynchus mykiss*).

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those reported by the study authors.

The study author reported that in the absence of any mortality or effects in an acute toxicity test (Huntingdon Research Centre Ltd. Report No. AGR 63©/891282), the exposure level selected for the (current) prolonged test was based on the stated limit of aqueous solubility of Rimon Technical, and that to ensure that the fish were exposed to the test material at its limit of solubility, the selected value intentionally exceeded the limit of solubility (p. 9).

The nominal 10 μg/L test samples were also filtered and centrifuged. The test concentration samples that were filtered ranged from <0.25 to 0.63 μg/L. The study authors stated that the test material (Rimon Technical) adsorbed onto plastic in previous studies and may have adhered to the filter papers. The test samples were then centrifuged at 3000 rpm for 15 minutes. The test concentration samples that were centrifuged ranged from 1.62 to 4.40 μg/L. The mean measured test concentrations were based on the unfiltered samples.

The limit of aqueous solubility of Rimon Technical was 2.59 μg/L, based on the centrifuged samples.

With the exception of Day 7, stability across all sampling periods exceeded the acceptable level of 70% of initial concentrations.

G. CONCLUSIONS:

The study is scientifically sound; however, it was not designed to fulfill any current U.S. EPA FIFRA guideline. This study is therefore classified SUPPLEMENTAL, as it provides useful information on the 28-day sub-chronic toxicity of Rimon Technical (Novaluron) to the Rainbow trout (*Oncorhynchus mykiss*).

28-Day Survival:
LC_{50}: >6.16 μg a.i./L 95% C.I.: N/A
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

Toxic Effects:
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

28-Day Wet Weight:
NOEC: >6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

28-Day Length:
NOEC: 6.16 μg a.i./L
LOEC: >6.16 μg a.i./L

Most sensitive endpoint(s): None

III. REFERENCES:

OECD Guidelines for testing of Chemicals. "Fish Prolonged Toxicity Test: 14-day study". Procedure 204, adopted 4 April 1984.

Huntingdon Life Sciences Report No. MAK418/970332. Rimon (Pure): Determination of physico-chemical properties.

Huntingdon Research Centre Ltd Report No. AGR 63(c)/891282. The Acute toxicity of GR 572 Technical to Rainbow trout.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Wet weight

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   Standard Two-Sample t-Test

data: neg control: V1 in DS1 , and solvent control: V2 in DS1
t = -0.6583, df = 18, p-value = 0.5187
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.7964023  0.4164023
sample estimates:
mean of neg control: 3.409
mean of solvent control: 3.599


   Standard Two-Sample t-Test

data: pooled control: V1 in DS1 , and 6.16 ug/L: V3 in DS1
t = 0.0572, df = 28, p-value = 0.9548
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.5919791  0.6259791
sample estimates:
mean of pooled control: 3.504
mean of 6.16 ug/L: 3.487
```